Pestic. Sci. 1998, **54**, 300–322

Extended Summaries
9th International Congress of Pesticide Chemistry

The following are extended summaries based on material from poster presentations at the 9th International Congress of Pesticide Chemistry, organised by the International Union of Pure and Applied Chemistry (IUPAC) and held in London, UK on 2–7 August 1998. The summaries published here are entirely the responsibility of the authors and do not necessarily reflect the views of the Editorial Board of Pesticide Science.

Mode of Action of Insect Growth Regulators in Lepidopteran Tissue Culture

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Abstract: Insect growth regulators (IGRs) have been proposed as agents for the control of insect pests. These compounds disrupt the normal development of insects by mimicking juvenile hormone and the molting hormone, 20-hydroxyecdsyone, or by interfering with chitin synthesis. The effectiveness and selectivity of IGRs provide new tools for integrated pest management. The simultaneous advances in the chemistry of IGRs and the ability to study insect tissues in culture, have led to research on the mode of action of IGRs *in vitro*. *Plodia interpunctella* and *Spodoptera frugiperda* have been used to examine the effects of IGRs on wing imaginal discs in organ culture, as well as in hormonally responsive cell lines established from wing imaginal discs of these species. Our research has focused on the action of ecdysteroid mimics, chitin synthesis inhibitors and juvenile hormone mimics. The effects of the IGRs on chitin synthesis, uptake of amino-sugars, and cellular proliferation were studied in tissue culture. The results demonstrate the effectiveness of using organ cultures and hormonally responsive cell lines for investigating IGRs at the cellular and tissue level. © 1998 Society of Chemical Industry

Pestic. Sci., **54**, 000–000 (1998)

Key words: tebufenozide; methoprene; fenoxycarb; chlorfluazuron; diflubenzuron; insect growth regulators; juvenile hormone mimics

The use of tissue culture techniques to study insect physiology was first described in a publication on spermatogenesis by Goldschmidt in 1915,[1] but, substantial interest in such techniques was not evident until the 1960s. Even in 1983, Marks pointed out that the 'use of organ and cell cultures to study the mode of action of various agricultural and industrial chemicals is a new field with considerable potential'.[2] While there was rapid progress in the utilization of organ cultures for studying the action of insect hormones, particularly ecdysteroids, there was little progress in using established cell lines for this purpose until the 1980s. Most insect cell lines at that time derived from embryonic tissues or the ovariole sheath. Thus, it was difficult to utilize continuous cell lines for studying hormonal effects, given the unknown specific source of the cells, and the lack of retention of any specialized properties by the dividing cells. This problem was overcome with establishment of cell lines from wing imaginal discs which retained their ability to respond to ecdysteroid hormones.[3,4] Several reviews have focused on the advantages of utilizing cell lines as well as organ cultures for investigating hormonal action in insects.[5–7] With increasing use of organ and cell cultures for this purpose, it became apparent that these same systems could be used to study the action of insect growth regulators (IGRs) which either mimic insect hormones or otherwise interfere with developmental processes.

Chitin, an essential component of insect cuticle, is central to the structural integrity of the exoskeleton, and thus provides a significant target for selective insecticides. Benzoylphenyl ureas represent one class of IGRs that affect chitin synthesis,[8,9] but it has not been possible with in-vivo approaches to determine their mode of action.[10] In this laboratory we have tested the hypothesis that benzoylphenyl ureas affect the transport of precursors of chitin into the cells. We first demonstrated that 20-hydroxyecdysone stimulated uptake of GlcNAc and other amino sugars in the IAL-PID2 cell line-derived imaginal discs of *Plodia interpunctella* (Hübner).[11] Subsequently, using both cultured imaginal discs and the PID2 cells to test whether benzoylphenyl

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 Pestic. Sci. 0031–613X/98/$17.50. Printed in Great Britain

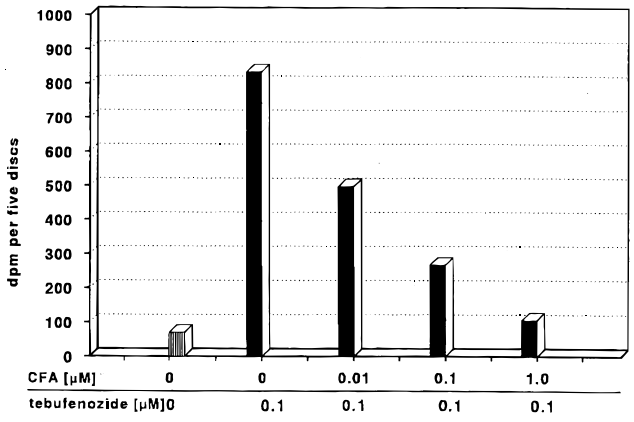

Fig. 1. Effects of various concentrations of chlorfluazuron (CFA) on tebufenozide-stimulated incorporation of [^{14}C]-GlcNAc into chitin by cultured wing imaginal discs of last larval instar *Spodoptera frugiperda* (0.1 μM tebufenozide; 48 h).

ureas prevented ecdysteroid-induced uptake of [^{14}C]-GlcNAc, we found that neither diflubenzuron nor teflubenzuron (which inhibit chitin synthesis in *P. interpunctella* wing imaginal discs) inhibited transport of GlcNAc into either the imaginal discs or the cell line.[12] The effects of chitin synthesis inhibitors on chitin precursor uptake was tested further in *S. podoptera frugiperda* (J. E. Smith). Diflubenzuron, teflubenzuron and chlorfluazuron all inhibited chitin synthesis in imaginal discs of this species, but did not block transport of GlCNAc into the PID2 cells. However, exposure of imaginal discs to chlorfluazuron for as little as 15 minutes inhibited ecdysteroid-dependent chitin synthesis when measured three days later.[13] This brief but effective treatment with inhibitor provided an opportunity to discern whether chlorfluazuron selectively inhibited chitin synthesis, or whether there were diverse effects, as observed with in-vivo experiments. An ultrastructural analysis of chlorfluazuron-treated *S. frugiperda* imaginal discs showed that the effects were selective for chitin synthesis, and that there was no influence on other morphogenetic and cellular events in the ecdysteroid-treated imaginal discs.[14] It is concluded from these tissue culture experiments that the site of inhibition for benzoylphenyl ureas resides in the movement of amino-sugar molecules to the epithelial surface and/or their assemblage into chitin on the cell surface.

The development of insect organ and cell culture assays to investigate the actions of the hormones 20-hydroxyecdysone and juvenile hormone, has provided opportunities to examine the effects of IGRs that mimic these hormones. Particular success has been obtained in recent years in investigations of a series of non-steroidal ecdysteroid agonists, the dibenzoyl hydrazines. The first compound announced in this group, RH-5849, was tested in a *Drosophila* cell line as well as *in vivo* in Lepidoptera.[15,16] Within a short time the organ culture and cell lines from *P. interpunctella* that had been developed in our laboratory were used to show that RH-5849 mimicked 20-hydroxyecdysone by stimulating evagination and chitin synthesis in cultured imaginal discs, and by increasing GlcNAc uptake in PID2 cells and decreasing cell proliferation.[17,18] Moreover, Wing and Aller showed that RH-5849 competed with ponasterone-A in binding studies of PID2 cell nuclear extracts, thus indicating that the compound bound to ecdysteroid receptors.[19] Smagghe and Degheele showed that a more potent ecdysteroid agonist, RH-5992 (tebufenozide) stimulated evagination in cultured wing imaginal discs of *Spodoptera exigua* (Hübner), while at the same time competing with ponasterone-A for binding to ecdysteroid receptor sites.[20] Using cultured wing imaginal discs of *S. frugiperda*, we found that 0.1 μM tebufenozide was as effective as 2.0 μM 20-hydroxyecdysone in stimulating incorporation of [^{14}C]-GlcNAc into chitin. In other experiments tebufenozide was an effective stimulator of [^{14}C]GlcNAc incorporation into chitin by the cultured imaginal discs. This assay was used successfully to demonstrate dose-dependent inhibition of chitin synthesis by chlorfluazuron (Fig. 1).

The use of lepidopteran tissue culture for studying juvenile hormone (JH) and its mimics has been more difficult than has the in-vitro approach to research on ecdysteroids or chitin synthesis inhibitors. Early work on *P. interpunctella* imaginal discs indicated that JH-I could prevent ecdysteroid-stimulated cuticle formation in cultured wing imaginal discs.[21] However, the dose of JH required to prevent cuticle formation was high. Therefore, subsequent studies involved intact larvae which were first treated with JH, and subsequently the imaginal discs were dissected out and cultured *in vitro* to evaluate their ecdysteroid responsiveness.[22] Recently, progress has been made in utilizing the PID2 cell line to assay for JH and its mimics. In these experiments JH-I, JH-III, and the JH mimics, fenoxycarb and methoprene, depressed cell proliferation in the PID2 line in a dose-dependent manner (Oberlander, Shaaya and Leach, unpublished). Thus, there is substantial progress in utilizing in-vitro culture of lepidopteran tissues to investigate the action of the ecdysteroid mimics, chitin synthesis inhibitors and JH mimics in their capacity as IGRs for control of economically important insects.

Acknowledgements

The authors thank Mr C. E. Leach for technical support throughout these studies.

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Enantioselective Catalysis for Agrochemicals: Synthetic Routes to (*S*)-Metolachlor, (*R*)-Metalaxyl and (α*S*,3*R*)-Clozylacon

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Abstract: The application of enantioselective catalytic methods for the technical preparation of chiral agrochemicals is illustrated for three active ingredients of the acylanilide type. The key step for the technical synthesis of the herbicide (*S*)-metolachlor is the enantioselective hydrogenation of an imine intermediate using a novel iridium ferrocenyldiphosphine catalyst with an unprecedented high activity and 80% ee. (*R*)-metalaxyl and (α*S*,3*R*)-clozylacon were synthesized via the enantioselective hydrogenation of corresponding enamide precursors with Rh and Ru/binap catalysts with >95% and 99% enantiomeric purity, respectively. © 1998 Society of Chemical Industry

Pestic. Sci., **54**, 000–000 (1998)

Key words: Chiral acyl anilides; chiral switch; enantioselective hydrogenation; (*R*)-metalaxyl; (*S*)-metolachlor; (α*S*, 3*R*)-clozylacon

1 Introduction

The biological properties of chiral agrochemicals are often strongly related to the absolute configuration.[1] As a consequence, the technical synthesis of pure or enriched enantiomers is of growing importance in the modern agrochemical industry. A promising technology for very efficient asymmetric syntheses is the application of chiral catalysts but, in the end, the best overall synthesis will be chosen for commercial production. For a technically feasible catalytic process, high enantioselectivity is not the only prerequisite; other factors such as catalyst productivity, catalyst activity, catalyst stability, availability and quality of the starting material, etc., can be even more important from a production point of view.[2] Catalyst productivity, expressed

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